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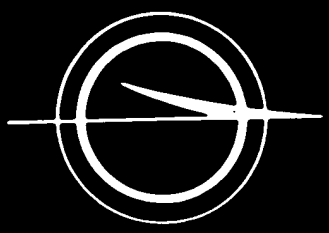

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Advances in the Applications of Monoclonal Antibodies in Clinical Oncology

Guest Editor

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Fusion Protein Mediated Prodrug Activation (FMPA) In Vivo

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ABSTRACT

A two component system, consisting of a fusion protein and an appropriate prodrug, suited to perform selective tumor therapy in vivo, is presented. The fusion protein, owing to its humanized carcinoembryonic antigen (CEA)-specific variable region, specifically binds to CEA-expressing tumors and has an enzymatic activity comparable to human β-glucuronidase. The prodrug is a nontoxic glucuronide-spacer-derivative of doxorubicin decomposing to doxorubicin by enzymatic deglucuronidation.

In vivo studies in nude mice bearing human CEA-expressing tumor xenografts revealed that 7 d after injection of 20 mg/kg fusion protein, a high specificity ratio (> 100:1) was obtained between tumor and plasma. Injection of 250 mg/kg of prodrug at d 7 resulted in tumor therapeutic effects superior to conventional chemotherapy without any detectable toxicity. These superior therapeutic effects that were observed using established human tumor xenografts can be explained by the approx 10-fold higher drug concentrations found in tumors of mice treated with fusion protein and prodrug than in those treated with the maximal tolerable dose of drug alone.

Index Entries: FMPA; CEA-expressing tumor; tumor therapy.

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INTRODUCTION

The major limitations of conventional chemotherapy are its lack of tumor selectivity resulting in high toxicity as well as generation of multidrug resistant tumor cells under the influence of long-term treatment with insufficient drug concentrations at the tumor site (for review, *see* ref. *1*). To overcome these problems of toxicity and multidrug resistance, several groups tried to develop antibody enzyme conjugates that ideally shall activate untoxic prodrugs to toxic drugs in high concentrations at the tumor site only (for review, *see* ref. *2*).

To get these site-specific activation systems working at least two or preferably three steps are needed (*3*). The first step includes the injection of a tumor-selective antibody enzyme conjugate into the tumor-bearing individual. After an appropriate localization phase of 1 or 2 d, a second antibody directed to the enzyme is injected to clear the antibody enzyme conjugate from the plasma. Thereafter an untoxic prodrug that can be cleaved to a cytotoxic drug by the enzyme moiety of the antibody enzyme conjugate localized at the tumor is injected.

The whole procedure as shown by studies in nude mice and by preliminary clinical trials generates superior therapeutic effects in comparison to conventional chemotherapy (*4*). Nevertheless, the high immunogenicity of the antibody enzyme conjugates, which consist of murine monoclonal antibodies chemically linked to xenogeneic enzymes, does not allow repetitive application of the conjugate leading to a limitation of the therapy. Furthermore, the insufficient clearance of the antibody enzyme conjugates and the inappropriate plasma stability of the prodrug result in high concentrations of drug in the plasma. This deficiency causes a significant toxicity to nontumorigenic tissues (*4*).

To reduce the problem of immunogenicity our group has generated a fusion protein consisting of a humanized CEA-specific binding region and human β-glucuronidase using recombinant DNA technology (*5*). The immunological and enzymological efficiency parameters of the fusion protein as well as its protein-chemical characteristics were extensively investigated in vitro (*5*).

In the present study we will present information concerning the pharmacokinetics of the fusion protein after iv injection in human tumor-bearing nude mice and its potential to activate an appropriate glucuronyl-spacer-doxorubicin prodrug (*6*) in vivo. Furthermore, the therapeutic efficiency of the concept of fusion protein mediated prodrug activation (FMPA) on the growth of established human tumor xenografts in nude mice will be reported.

MATERIALS AND METHODS

Animals

Female nude mice were obtained from Hagemann GmbH (Sulzfeld, Germany; strain CD-1 *nu/nu*) with a weight of about 18 g. Animals were

housed in macrolon cages and fed with tap water and standard diet (Ssniff NM, Versuchstierdiäten, Soest, Germany) ad libitum. Light/darkness period was 12 h/12 h. Room temperature was 26–27°C, relative humidity was 65–70%.

Tumor Implantation

Implantation was performed by subcutaneous injection of 0.2 mL of a cell suspension containing 2×10^6 tumor cells in the right dorsal region of nude mice on d −26.

Analysis of Therapeutic Activity

CD-1 *nu/nu* mice (seven animals/group) bearing sc growing LoVo colon carcinomas or Mz-Sto-1 stomach carcinomas received an iv bolus injection of 20 mg/kg of fusion protein on d -8. On d 0 these mice received an infusion of prodrug (250 mg/kg) over a period of 5 min. Other non-fusion protein-treated animals received on d 0 a 5-min infusion of physiological saline, prodrug (250 mg/kg), or doxorubicin (10 mg/kg) alone. Tumor growth was monitored over time by measurement of two perpendicular tumor diameters. Mean relative tumor areas were calculated from tumor diameters measured at individual days divided by tumor diameters measured at start of therapy (d 0). For treated groups percentage T/C values at individual days were calculated according to:

[mean relative tumor area (treated group)/mean relative tumor area (control group)] × 100

Preparation of Organs for Fusion Protein Quantification

Animals were sacrificed at the time intervals indicated; organs were removed and weighed. After addition of 2 mL 1% bovine serum albumin (BSA) in PBS, pH 7.2, organs were homogenized using a 5 mL potter homogenizer (Fa. Braun). Homogenates were adjusted to pH 4.2 and centrifuged at 16,000*g* for 30 min. The clear supernatants were neutralized using 0.1*N* NaOH and evaluated for functionally active fusion protein content in the Organ Enzyme Activity Test (OEAT).

OEAT

The amounts of fusion protein extracted from animal tissues and plasma were determined using the OEAT. Goat antihuman κ antibody (Fa. Southern Biotechnology Associates) was attached to individual wells of round bottom microtiter plates (Nunc, No. 4-60445). After blocking of unspecific sites with 1% casein, pH 7.2, 50 μL of fusion protein containing sample is added and incubated for 30 min at room temperature (RT). After extensive washing of the plates, 50 μL of a 2.5 m*M* 4-methylumbelliferyl-β-D-glucuronide solution in 200 m*M* of sodium acetate buffer, pH

5.0, was added and incubated for 24 h at 37°C. The reaction was stopped using 100 μL of 0.2*M* glycine + 0.2% of sodium dodecyl sulfate (SDS), pH 11.1. Fluorogenic units (FU) were translated to fusion protein concentrations based on appropriate standard dilution curves determined with purified fusion protein (*5*).

Immunohistochemical Methods

Fusion protein present in tissues of mice or human tumor xenografts were detected using an alkaline phosphatase (AP)-labeled goat antihuman *x* antibody or an AP-labeled antihuman β-glucuronidase monoclonal antibody (MAb) as described (*5*).

Analysis of Prodrug and Drug in Plasma and Organs

Blood (200 μL) collected by retroorbital punction (rats, mice) or by withdrawal from a venous catheter (monkeys) was diluted with 40 μL citrate containing 10 m*M* saccharic-1,4-lactone monohydrate (saccharolactone). Plasma was prepared by centrifugation (10 min, 1000*g*) and further diluted (1:7) with phosphate buffer, pH 6.0, containing 10 m*M* of saccharolactone and 0.01% of BSA.

After weighing of organs and tumor tissues, 20 m*M* of phosphate buffer, pH 3.0, containing 10 m*M* of saccharolactone, and 0.01% of BSA was added (230 mg tissue/770 μL buffer). Tissues were homogenized by means of an ultraturrax (1 min, 0°C). 200 μL of homogenate were mixed with 40 μL silver nitrate (3.3%) and 160 μL acetonitrile, shaken (30 min), and centrifuged (12,000*g*, 5 min). Prior to high-pressure liquid chromotography (HPLC) analysis, the supernatant (100 μL) was diluted with phosphate buffer, pH 6.0 (*see above*).

HPLC Analysis

The HPLC apparatus consisted of an autosampler (Abimed, model 231), an automatic sample extraction system (AASP, Varian) equipped with minicartridges containing C 18 reversed phase silica gel (Analytichem), a gradient pump (Gynkotek, model 480), and a fluorescence detector (Shimazdu RF 535, excitation: 495 nm, emission: 560 nm). Before sample injection, the minicartridges were preconditioned with 2.5 mL methanol and 1.5 mL 20 m*M* phosphate buffer, pH 6.0. After injection of the sample (350 μL) the cartridges were washed with 1.5 mL phosphate buffer, pH 6.0. Analytes retained on the reversed phase silica gel, were then eluted by valve switching and connection of the minicartridges to the mobile phase. Chromatography was performed on reversed phase material (Nucleosil C 18, 5 μm particle size, 120 mm length, 4.5 mm ID) and gradient elution. Elution was done by a gradient composed of 2 components (A: 20 m*M* phosphate, pH 3.0, B: acetonitrile). The gradient was run with the following time-concentration profile:

Table 1
In Vivo Distribution of Fusion Protein[a]

	μg Fusion protein localized per g organ, OEAT							
Time, h	Tumor, Mz-Sto-1	Plasma	Liver	Gut	Kidney	Lung	Heart	Tumor, LXF-529
0.05	3.0	456.2	61.4	6.5	35.6	78.0	60.1	9.1
1.0	4.9	199.9	26.1	8.6	17.3	34.0	19.5	n.d.
3.0	5.7	122.0	14.8	3.5	6.5	8.0	2.5	1.1
5.5	3.8	84.9	8.4	3.8	7.7	9.0	2.9	n.d.
24.0	4.7	19.0	2.1	0.6	2.5	2.0	0.5	0.190
168.0	0.19	<0.001	0.003	<0.001	<0.001	<0.002	<0.001	<0.001

[a]Values are means out of three organs per time (SD < ± 50%).

0 min: 75% A, 25% B;
20 min: 25% A, 75% B;
30 min: 25% A, 75% B.

Before starting the next run the column was allowed to equilibrate at starting conditions for 5 min.

Enzyme Kinetics

K_m and V_{max} values were calculated using the computer program GraFit (Erithacus Software Ltd., Staines, UK).

RESULTS

In Vivo Distribution and Retention of Fusion Protein

Single iv injection of 400 μg of fusion protein in nude mice bearing established CEA-expressing (Mz-Sto-1) or CEA-negative (LXF-529) human tumor xenografts resulted in a selective retention of functionally active fusion protein during a 7-d period in CEA-expressing tumors (Table 1). At d 7 approx 200 ng of functionally active fusion protein were found per gram of the CEA-expressing human stomach carcinoma xenograft (Mz-Sto-1).

In contrast, fusion protein concentrations per gram of a CEA-negative large cell lung carcinoma xenograft (LXF-529) were below 1 ng at d 7. Comparable low fusion protein concentrations were obtained in normal nude mouse tissues at d 7 (Table 1). These data demonstrate that 7 d after iv application an in vivo specificity ratio ≥ 100 between CEA-expressing tumor and CEA-negative tumor or organ were obtained. Similar specificity ratios as well as absolute fusion protein concentrations were obtained using a second CEA-expressing colon carcinoma xenograft (LoVo) in an independent set of experiments.

Kinetics of Fusion Protein's Tissue Penetration

In addition to the quantitative data generated using the OEAT that define the amounts of functionally active fusion protein per gram tissue, a semiquantitative immunohistochemical method was applied, allowing the visualization of the fusion protein on cryopreserved tissue sections in its microenvironment. Already 3 min after iv injection of 400 μg of fusion protein in nude mice bearing established CEA-positive Mz-Sto-1 xenograft, a heterogenous staining of certain areas in the tumor thin sections was seen (Fig. 1c). The strength of staining slightly increased up to 24 h after injection, however remaining heterogeneous (Fig. 1c–f). Thereafter a decrease of staining intensity was observed that, however, at d 7, is still significantly above background level (Fig. 1g).

These semiquantitative immunohistochemical staining reactions are in agreement with the quantitative data presented above using the OEAT (Table 1). They clearly demonstrate that the fusion protein selectivity binds to CEA-positive human tumor xenografts remaining there as a functionally active molecule for at least 7 d.

Plasma Stability of Prodrug and In Vitro Cleavage

After determination of the fusion protein's in vivo distribution, tumor retention and tissue penetration, the prodrug's plasma stability and pharmacokinetics were evaluated in order to be able to combine the two components adequately. The prodrug (*N*-[4-β-glucuronyl-3-nitro-benzyloxycarbonyl]doxorubicin) (*6*) (Fig. 2) is very stable under in vitro conditions in human, rat, or mouse plasma. After 50 h of incubation of the prodrug at 100 μg/mL, maximally 20% of the prodrug is cleaved (Fig. 3). Addition of 1.6 μg/mL of fusion protein to a solution of prodrug (335 μg/mL) results in a quick disappearance of prodrug owing to deglucuronidation (Fig. 4). The resulting doxorubicin spacer derivative spontaneously decomposes to doxorubicin (Fig. 4). Based on a number of similar in vitro cleavage experiments in buffer at pH 7.2, K_m and V_{max} were calculated to be 1.3 m*M* and 0.635 nmol/min·μg at 37°C.

Pharmacokinetics of Prodrug

Plasma half-life of prodrug was determined after a single bolus iv injection of 250 mg/kg prodrug in CD1 *nu/nu* mice as well as of 50 mg/kg prodrug in Macaca fascicularis monkeys and in CD rats (Table 2). Prodrug and potentially arizing drug concentrations were determined using reversed phase HPLC followed by data analysis in the HoeRep computer program.

In all three animal systems, pharmacokinetics of the prodrug fit into a two compartment model with an elimination half-life ($t_{1/2}\beta$) between 77–198 min and a distribution half-life ($t_{1/2}\alpha$) between 3–19 min. The concentrations of free doxorubicin (drug) detectable in plasma of these prodrug-

treated animals were below 0.05% of the respective prodrug concentration determined at the individual measurements. These pharmacokinetic studies support the previous finding that the prodrug is not cleaved to a significant extent in plasma of rodents and higher mammals.

Maximal Tolerable Dose (MTD) of Prodrug and Drug In Vivo

The MTD of prodrug and drug was determined by 5 min iv infusions of increasing dosages of prodrug or drug in CD1 *nu/nu* mice. MTD of the drug was 12 mg/kg, that of the prodrug was >1600 mg/kg, if applied in doses of 800 mg/kg in 6-h intervals. Applications of a single dose larger than 800 mg/kg were not possible because of the water solubility of the prodrug (30 mg/mL). According to these data, the prodrug is at least 80-fold less toxic in vivo than the drug.

Therapeutic Efficacy

Based on the in vivo distribution and tumor retention data generated using the fusion protein as well as on the pharmacokinetics and the MTD data obtained for the prodrug, therapy experiments were performed. Seven days after injection of 20 mg/kg of fusion protein into nude mice bearing established CEA-expressing human tumor xenografts (tumor diameter ≈5 mm) (LoVo) 250 mg/kg of prodrug were infused. In addition, separate groups of animals were treated with prodrug alone, doxorubicin, or physiological saline.

Therapeutic efficacy was documented by monitoring tumor growth (Fig. 5). Significant growth retardation with partial regression was obtained only in those animals receiving fusion protein and prodrug (26% T/C). Prodrug alone (68% T/C) or the MTD of doxorubicin (61% T/C) had no significant antitumoral effect against this particular tumor. Comparable data were obtained using another CEA expressing human tumor xenograft (Mz-Sto-1, stomach carcinoma).

DISCUSSION

This preclinical study demonstrates that appropriate in vivo application of a tumor selective humanized fusion protein and a nontoxic prodrug generates tumor therapeutic effects superior to conventional chemotherapy (Fig. 5).

These impressive therapeutic effects were obtained in human tumor xenograft models (LoVo, Mz-Sto-1) resistant to conventional chemotherapy and with a single cycle of fusion protein and prodrug. Owing to the presumably low immunogenicity of the fusion protein consisting of a humanized CEA-specific binding region and human β-glucuronidase,

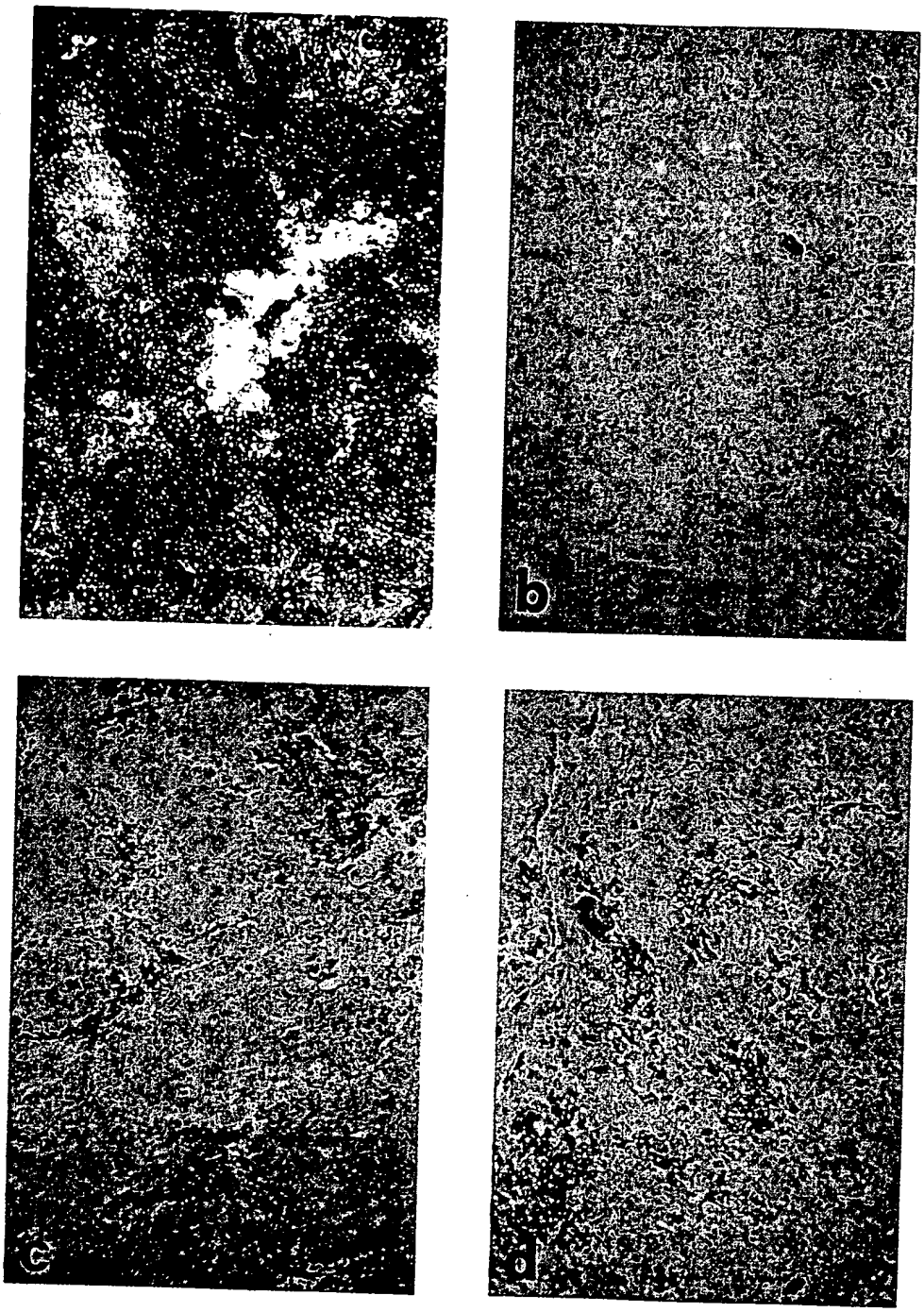

Fig. 1. Photograph showing cryosections of Mz-Sto-1 xenografts stained with AP-labeled goat antihuman κ antibody (second antibody): (**A**) Strong staining observed owing to addition of 1 μg/mL fusion protein followed by second antibody (positive control). (**B**) No staining observed in untreated xenograft with

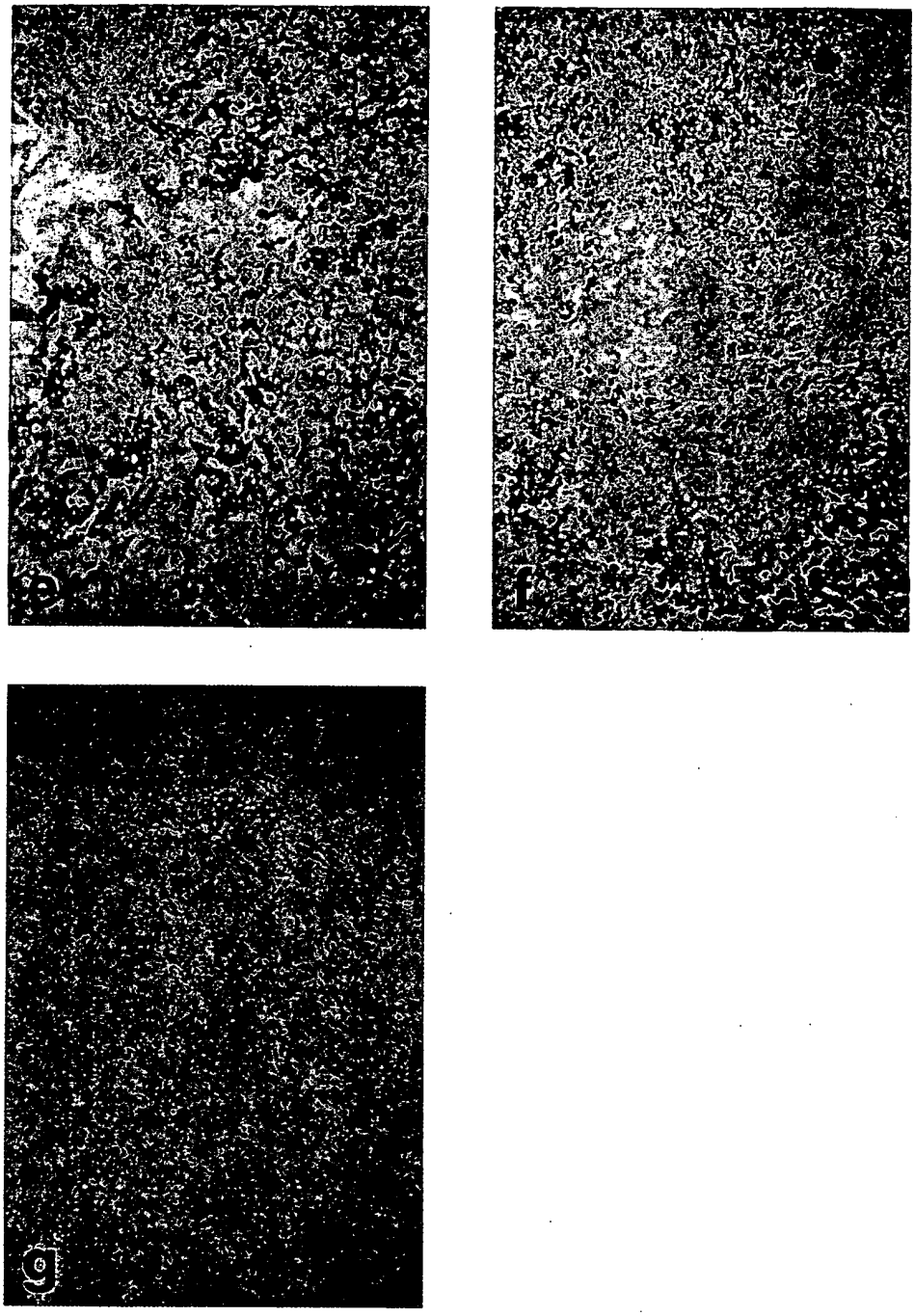

Fig. 1. (*cont'd*). second antibody (negative control). (C,D,E,F,G) Heterogeneous staining observed 3 min, 3 h, 6 h, 24 h, 7 d after iv application of 400 μg fusion protein followed by detection with second antibody.

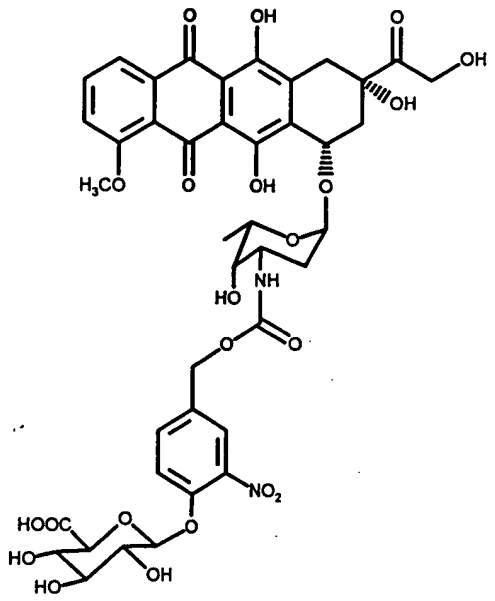

Fig. 2. Chemical structure of prodrug (*N*-[4-β-glucuronyl-3-nitro-benzyloxycarbonyl]-doxorubicin).

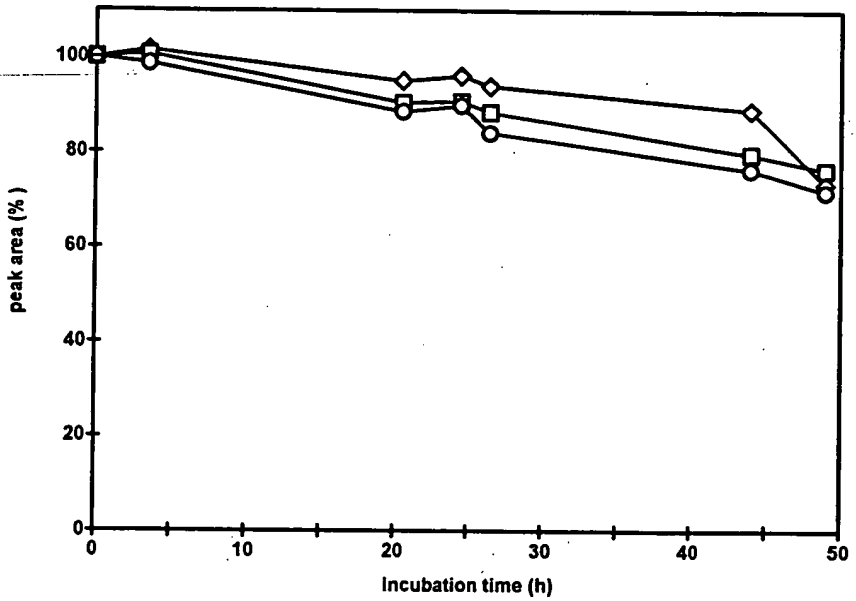

Fig. 3. Stability of prodrug in human (◇), mouse (□), or rat (○) plasma. Citrate-buffered plasma obtained from humans, mice, or rats was added to a solution of prodrug (200 μg/mL in 100 m*M* phosphate buffer, pH 7.35) (1:1) and incubated for various times at 37°C. Prodrug concentrations were analyzed according to the procedures described in Materials and Methods. The data shown represent percentages of prodrug peak areas from the total peak area (value obtained at 0 min = 100%).

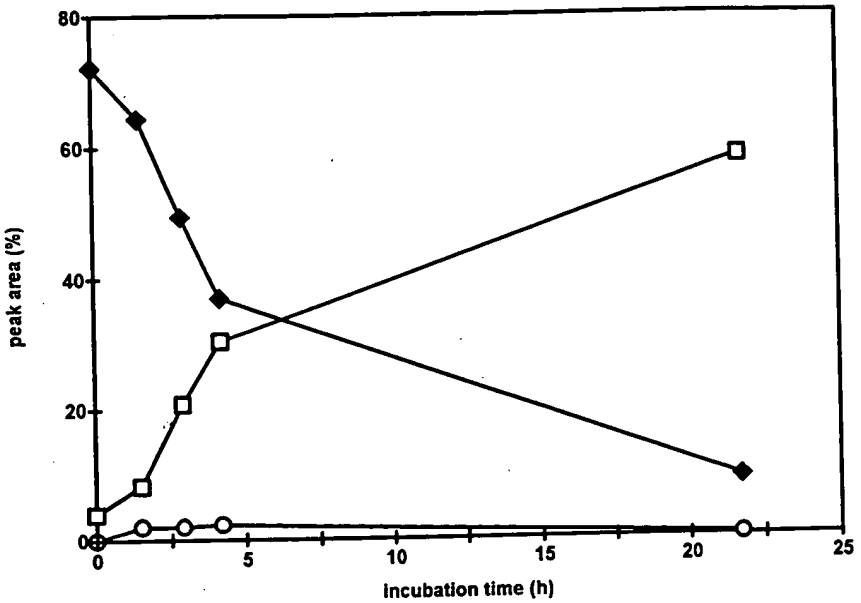

Fig. 4. Kinetics of cleavage of prodrug by fusion protein in vitro. Prodrug (335 μg/mL in 20 m*M* phosphate buffer, pH 7.2) was incubated with fusion protein (1.6 μg/mL) at 37°C. HPLC-analysis of drug concentrations was performed according to the procedures described in Material and Methods. (◆) prodrug; (□) doxorubicin; (○) doxorubicin-spacer.

Table 2
Pharmacokinetics of Prodrug in Different Species

Species	Application	Dose, mg/kg	$t_{1/2}\alpha$, min	$t_{1/2}\beta$, min
CD1 *nu/nu* mice	iv bolus	250 mg/kg	18.75	82.71
	iv 5′ infusion	250 mg/kg	11.74	114.5
CD rats	iv bolus	50 mg/kg	14.11	198.56
Macaca fascicularis monkeys	iv bolus	50 mg/kg	3.25	77.45

repetitive applications of fusion protein should be possible. Furthermore, the prodrug dose used in our therapy experiment (250 mg/kg) is only approx 1/6 of the MTD of the prodrug. Therefore, repetitive treatment cycles probably can be applied in patients.

The application of repetitive treatment cycles does not seem to be possible without immunosuppressive therapy in systems containing mouse MAbs chemically linked to xenogeneic enzymes (7). In addition, to be effective some xenogeneic antibody-enzyme conjugate systems (3) need as a third component the injection of a galactosilated antienzyme MAb to clear the xenogeneic antibody-enzyme-conjugate from the plasma

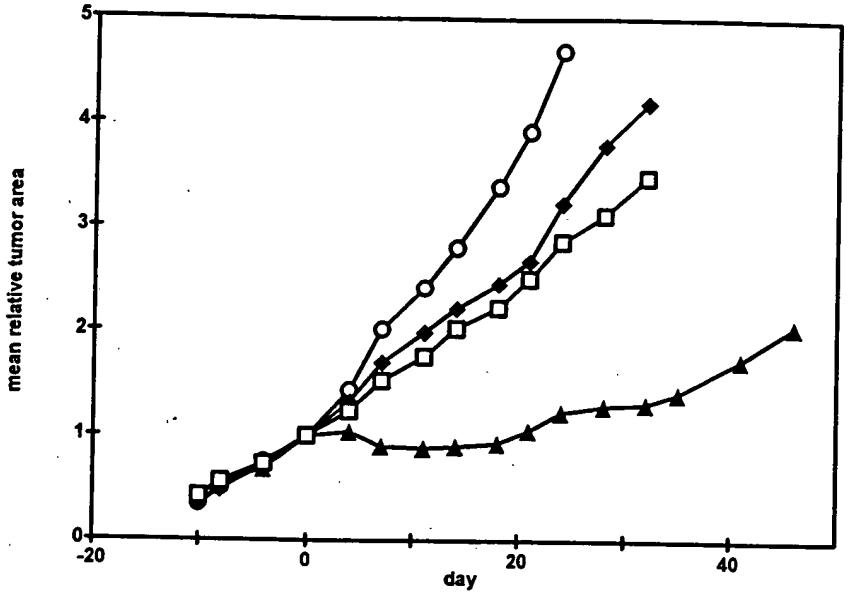

Fig. 5. Therapeutic efficacy of the combined application of fusion protein and prodrug in nude mice bearing sc growing LoVo colon carcinomas. Tumor bearing nude mice were treated with fusion protein on d −8 (iv bolus) followed by a 5 min infusion of prodrug (250 mg/kg) on d 0, at which time the mean tumor area was about 35 mm². Other groups received 5-min infusions of either physiological saline, prodrug (250 mg/kg), or doxorubicin (10 mg/kg) alone. (○) control; (▲) fusion protein + prodrug; (◆) prodrug; (□) doxorubicin.

before prodrug injection. Such a clearing step is not needed in our system, because the fusion protein is quickly eliminated from plasma, mainly by internalization into liver cells (Table 1). This efficient internalization mechanism is responsible for the very high ratios (> 100:1) of functionally active fusion protein between tumor and plasma or organs (Table 1). Despite its high mol wt (250 kDa) and the known diffusion barriers in solid tumors (*8*), the fusion protein is able to penetrate human tumor xenograft tissues (Fig. 1). Although the staining reaction after iv application of the fusion protein remains heterogeneous (Fig. 1) the amounts of functionally active fusion protein in the tumor at d 7 (Table 1) are still suitable to activate the prodrug in vivo very efficiently (*6*) (Fig. 2). This is only possible because the prodrug has several advantageous characteristics, i.e., the high tolerability (> 1600 mg/kg), high plasma stability (Fig. 3), and nearly optimal cleavability by the fusion protein (Fig. 4). These favorable characteristics of the prodrug allow to inject relatively high amounts (250 mg/kg) to be able to reach half of the K_m of the human β-glucuronidase for a sufficient period of time in the tumor (data not shown). The resulting amounts of drug lead to impressive therapeutic effects without any detectable toxicity to the animals (Fig. 5). A preliminary analysis of prodrug and drug con-

centrations in the tumor reveals that drug concentrations are at least 1 log higher in the fusion protein plus prodrug group than those drug concentrations received after application of the MTD of doxorubicin. Taken together, the pharmacological and analytical data should be encouraging enough to develop our fusion protein mediated prodrug activation system clinically.

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REFERENCES

1. Ford, J. M. and Hait, W. N. (1990) *Pharmacol. Reviews* **42,** 155.
2. Senter, P. D., Wallace, P. M., Svensson, H. P., Vrudhula, V. M., Kerr, D. E., Hellström, I., et al. (1993) *Bioconjugate Chem.* **4,** 3.
3. Sharma, S. K., Bagshawe, K. D., Springer, C. J., Burke, P. J., Rogers, G. T., Boden, J. A., et al. (1991) *Disease Markers* **9,** 225.
4. Bagshawe, K. D., Sharma, S. K., Springer, C. J., Antoniw, P., Boden, J. A., Rogers, G. T., et al. (1991) *Disease Markers* **9,** 233.
5. Bosslet, K., Czech, J., Lorenz, P., Sedlacek, H. H., Schuermann, M., and Seemann, G. (1992) *B. J. Cancer* **65,** 234.
6. Jacquesy, J.-C., Gesson, J.-P., Monneret, C., Mondon, M., Renoux, B., Florent, J.-C., et al. Prodrogues glycosylées, leur procédé de préparation et leurs utilisation. Demande de brevet européen, EP 0 511 917 A1.
7. Bagshawe, K. D., Sharma, S. K., Springer, C. J., Antoniw, P., Rogers, G. T., Burke, P. J., et al. (1991) *Antibody, Immunoconjugates and Radiopharmaceuticals* **4,** 915.
8. Jain, K. R. (1987) *Cancer Res.* **47,** 3039.